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<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, 15/63, 5/10, C12Q 1/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/04821</b> <b>(43) International Publication Date:</b> 16 February 1995 (16.02.95)
<b>(21) International Application Number:</b> PCT/US94/08913 <b>(22) International Filing Date:</b> 3 August 1994 (03.08.94)  <b>(30) Priority Data:</b> 104,517 9 August 1993 (09.08.93) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 104,517 (CON) Filed on 9 August 1993 (09.08.93)  <b>(71) Applicant (for all designated States except US):</b> MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> GRAZIANO, Michael, P. [US/US]; 2239 Concord Road, Scotch Plains, NJ 07076 (US). BORKOWSKI, Doreen, A. [US/US]; 237 Edgewood Avenue, Westfield, NJ 07090 (US). CHICCHI, Gary, G. [US/US]; 64 Frost Avenue, East Brunswick, NJ 08816 (US). HEY, Patricia, J. [US/US]; 10 Mary Lane, Fanwood, NJ 07023 (US). STRADER, Catherine, D. [US/US]; 119 Morningside Road, Verona, NJ 07044 (US).		<b>(74) Common Representative:</b> MERCK & CO., INC.; Patent Dept., 126 East Lincoln Avenue, Rahway, NJ 7065 (US).  <b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HUMAN GLUCAGON-LIKE 1 PEPTIDE RECEPTOR		
<b>(57) Abstract</b>  The human GLP-1 receptor is cloned and used in an <i>in vitro</i> assay to screen for compounds that specifically bind to the human GLP-1 receptor, including compounds effective to reduce symptoms of diabetes. The invention includes the assay, the cloned human receptor used in the assay, an isolated human GLP-1 receptor free of other human proteins, and compounds identified through the use of this novel, cloned receptor, which selectively bind the human GLP-1 receptor.		

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TITLE OF THE INVENTION

## HUMAN GLUCAGON-LIKE 1 PEPTIDE RECEPTOR

BRIEF DESCRIPTION OF THE DRAWINGS

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Figure 1. Sequence of the human GLP-1 receptor. The 463 residue long amino acid sequence deduced from the cDNA sequence is shown in single letter code. The 337 base pair (bp) fragment (hGLP-1 fragment 1) isolated by degenerate PCR is underlined. Three consensus sequences for N-linked glycosylation are marked with diamonds.

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Figure 2. Comparison of the human and rat GLP-1 receptor cDNA sequences. Comparison of the human (top) and rat GLP-1 receptors was made using the GCG (Genetics Computer Group) Gap program. The 7 putative transmembrane domains are boxed.

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Figure 3. Displacement of [ $^{125}$ I] GLP-1 (7-36) amide binding to transfected COS-7 cells. COS-7 cells ( $7 \times 10^6$  cells) were transfected with 20  $\mu$ g of human GLP-1 receptor cDNA in pcDNA1/neo and membranes prepared and frozen from the cells. 27  $\mu$ g of membrane protein was incubated with 50 pM [ $^{125}$ I] GLP-1 (7-36) amide and the indicated concentrations of ligand. Data shown are means  $\pm$  S.E.M. of duplicate determinations and are representative of two experiments. Symbols: squares, GLP-1 (7-36) amide; triangles, glucagon; diamonds, gastric inhibitory peptide; circles, secretin.

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Figure 4. cAMP accumulation in transfected COS-7 cells. COS-7 cells ( $7 \times 10^6$  cells) were transfected with 100  $\mu$ g of human GLP-1 receptor cDNA. Cells were harvested and cAMP accumulation was determined as outlined in the Examples. Data shown are the mean  $\pm$  S.E.M. from triplicate determinations from a single experiment and are representative of three experiments. Symbols, triangles, GLP-1 7-36 amide; squares, glucagon.

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## BACKGROUND OF THE INVENTION

This is a continuation of U.S. Serial No. 08/104, 517 filed August 9, 1993, now pending.

5       Glucagon-like 1 peptide (GLP-1) is one of several hormones shown to potentiate glucose-induced insulin secretion. Such hormones, known as incretins, are produced in the gut, released in response to a meal, and their interaction with specific receptors on pancreatic islets causes insulin to be secreted in a glucose-dependent manner (H.-C. Fehmann, J. F. Habener, *Trends in Endocrinol. and Met.* 10       3, 158-163 (1992)). GLP-1 is produced by posttranslational processing of the proglucagon gene in intestinal L cells, through a biologically inactive 37 amino acid form [GLP-1 (1-37)] to either of two biologically active forms, GLP-1 (7-37) and GLP-1 (7-36) amide. These biologically active forms of GLP-1 are the most potent incretins 15       known, with effects on glucose-mediated insulin secretion being seen at concentrations as low as 10 pM. Infusion of GLP-1 (7-36) amide into patients with type II diabetes leads to increased secretion of insulin which occurs in an glucose-dependent fashion (D. M. Nathan, E. Schreiber, H. Fogel, S. Mojsov, J. F. Habener, *Diabetes Care* 15, 270- 20       276 (1992); M. Gutniak, C. Orskov, J. J. Holst, B. Ahren, E. S., *New Engl. J. Med.* 326, 1316-1322 (1992)). These data suggest that compounds that act via the GLP-1 receptor may be therapeutic in the treatment of type II diabetes.

25       GLP-1 receptors have also been described in lung (G. Richter, R. Goke, B. Goke, A. R., *FEBS Lett.* 267, 78-80 (1990)), adipose (C. Ruiz-Grande, C. Alarcon, E. Merida, I. Vaverde, *Peptides* 13, 13-16 (1992)); brain (N. S. Hoosein, G. R.S., *FEBS. Lett.* 178, 83-86 (1984)); and a gastric tumor cell line, HGT-1 (A. B. Hansen, C. P. Gespach, G. E. Rosselin, J. J. Holst, *FEBS Lett.* 236, 119-122 (1988)). 30       A cDNA encoding a GLP-1 receptor has recently been cloned from rat pancreatic islets (B. Thorens, *Proceedings of the National Academy of Sciences U.S.A* 89, 8641-8645 (1992)). This receptor has seven putative transmembrane domains and belongs to the superfamily of G protein coupled receptors. The GLP-1 receptor is most homologous to

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other members of a recently defined subclass of G protein coupled receptors that includes the receptors for glucagon and secretin. The GLP-1 receptor acts via stimulation of adenylyl cyclase to raise intracellular levels of cAMP (Fehmann *et al.*, *supra*).

The present invention pertains to the cloning, expression, and pharmacological characterization of a human GLP-1 receptor from the gastric tumor cell line HGT-1.

### SUMMARY OF THE INVENTION

The human glucagon-like 1 peptide (GLP-1) is cloned, expressed and used in an *in vitro* assay to screen for compounds that bind to the receptor, including compounds which specifically stimulate or inhibit the activity of the receptor. The invention includes the assay, the cloned receptor used in the assay, an isolated human GLP-1 receptor, cells expressing the cloned receptor, and compounds identified through the use of the cloned GLP-1 receptor which selectively bind to the human GLP-1 receptor, including specific agonists or antagonists of the receptor.

The human glucagon-like 1 peptide receptor of the present application was cloned from the gastric tumor cell line HGT-1. The cDNA clone encodes a protein of 463 amino acids. The predicted secondary structure places this receptor within the superfamily of seven transmembrane domain G protein coupled receptors. Transfection of the human GLP-1 receptor into COS-7 cells confers upon them high affinity binding for [<sup>125</sup>I] GLP-1 (7-36) amide. In membranes prepared from COS-7 cells transfected with the human GLP-1 receptor, the binding of [<sup>125</sup>I] GLP-1 (7-36) amide is inhibited with the rank order of potency GLP-1 (7-36) amide > glucagon > secretin; this is characteristic of a GLP-1 receptor. The human GLP-1 receptor expressed in COS-7 cells is functionally coupled to increases in intracellular cAMP. Incubation of COS-7 cells expressing the human GLP-1 receptor with GLP-1 (7-36) amide gives rise to a 4-fold increase in cyclic AMP over basal levels, with an EC<sub>50</sub> of 25 pM. Glucagon is

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200-fold less potent than GLP-1 as an agonist at the expressed human receptor.

#### DETAILED DESCRIPTION OF THE INVENTION

5           The human glucagon-like 1 peptide receptor (GLP-1) was identified, cloned and expressed in cell cultures by the instant inventors. A partial coding region for this receptor was generated by polymerase chain reaction technology (PCR). Degenerate oligonucleotides encoding amino acids present in the rat GLP-1 receptor were used to prime PCR  
10 reactions using human HGT-1 cDNA as a template. The predicted sized products were cloned and sequenced. Translation of the amplified cDNA yielded an open reading frame encoding a protein approximately 91% homologous to the rat GLP-1 receptor. This partial sequence was used to obtain a larger cDNA clone from a human HGT-1 library. The  
15 remaining receptor cDNA was obtained by a modification of the PCR-RACE (Rapid Amplification of cDNA ends) protocol (M. A. Frohman, M. K. Dush, G. R. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8998-9002 (1988)). A cDNA library was made from HGT-1 cDNA and the plasmid pcDNA I as outlined in Examples. PCR was performed using  
20 the HGT-1 library and primers to both the partial fragment of the human GLP-1 receptor and pcDNA I. A series of overlapping cDNA fragments were obtained and sequenced.

          The HGT-1 cDNA library and primers 5' TGGTGGATTCTGAAGTCC 3' (SEQ ID NO: 3) and 5'  
25 CCTGTGGTTTCACAAGAAGC 3' (SEQ ID NO: 4) were used in a PCR reaction to generate the complete receptor sequence (Figure 1). The open reading frame included in this sequence encodes a 463 amino acid protein that is approximately 91% identical to the rat GLP-1  
30 receptor sequence (Figure 2).

          The cloned human GLP-1 receptor, when expressed in mammalian cell lines including but not limited to, COS-7, CHO or L cells, is used to discover ligands that bind to the receptor and alter or stimulate its function. In addition, the cloned GLP-1 receptor enables quantitation of mRNA levels in human tissues, including the pancreas

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and gastrointestinal system, by RNase protection assays. For these purposes, a complete coding sequence of the receptor is provided.

5 The specificity of binding of compounds showing affinity for the GLP-1 receptor is shown by measuring the affinity of the compounds to membranes obtained from cells tranfected with the cloned GLP-1 receptor and membranes from tissues known to express GLP-1  
10 receptors. Expression of the cloned GLP-1 receptor, screening for compounds that inhibit the binding of radiolabeled GLP-1 (7-36) amide or compounds that stimulate cAMP production in these cells provides a rational way for selection of compounds and discovery of new compounds with predictable pharmacological activities.

Once the human receptor is cloned and expressed in a non-human cell line, such as COS-7 cells or CHO cells, the recombinant  
15 GLP-1 receptor is free of other human proteins. The membranes from the recombinant cells expressing human GLP-1 receptor are then isolated according to methods well known in the art and may be used in a variety of membrane associated receptor binding assays. One example of such an assay is described by Strader *et al.*, (*Proc. Natl. Acad. Sci. USA* **84**, 4384-4388, 1987). Generally, a compound of interest is used  
20 to compete with the binding of a known, quantifiable GLP-1 receptor ligand. Thus, radiolabeled [<sup>125</sup>I] GLP (7-36) amide or [<sup>3</sup>H]-GLP may be used for this purpose. Because of the ease of <sup>125</sup>I detection, [<sup>125</sup>I] GLP (7-36) amide is preferred for this purpose. By increasing the amount of unlabeled test compound, the labeled compound is competed  
25 off the receptor. From these experiments, IC<sub>50</sub> values for each test compound and receptor subtype is determined.

In addition, agonist ligands that activate the receptor may be detected by measuring the ability of added compounds to increase  
30 cAMP production mediated by the receptor expressed in COS-7 or CHO cells. cAMP can be measured directly by radioimmunoassay or by stimulation of adenylylcyclase in membranes prepared from the cells (Salomon, Y., Landos, C. and Rodbell, M. 1974. *Anal. Biochemistry*, Vol. **58**, 541-548) by methods that are well-known in the art.

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Thus, according to this invention, a method is provided for identifying compounds specific for the human GLP-1 receptor comprising the following steps:

- 5 a. Cloning the human glucagon-like 1 peptide (GLP-1) receptor;
- 10 b. Splicing the cloned GLP-1 receptor into an expression vector to produce a construct such that the GLP-1 receptor is operably linked to transcription and translation signals sufficient to induce expression of the receptor upon introduction of the construct into a prokaryotic or eukaryotic cell;
- 15 c. Introducing the construct into a prokaryotic or eukaryotic cell which does not express a human GLP-1 receptor in the absence of the introduced construct;
- 20 d. Incubating cells or membranes isolated from cells produced in Step c with a quantifiable compound known to bind to human GLP-1 receptors, and subsequently adding test compounds at a range of concentrations so as to compete the quantifiable compound from the receptor, such that an IC<sub>50</sub> for the test compound is obtained as the concentration of test compound at which 50% of the quantifiable compound becomes displaced from the receptor; and
- 25 e. Incubating cells or membranes from cells produced in Step c with test compounds in range of concentrations such that an ED<sub>50</sub> for the test compound is obtained. The ED<sub>50</sub> is defined as the concentration of compound which increases intracellular cyclic AMP to 50% of the maximal quantity of cAMP produced by interaction of the test compound with the human GLP-1 receptor.
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Whereas GLP-1 has been shown to increase secretion of insulin in diabetic patients, an agonist of the human GLP-1 receptor



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discovered as described above would be useful in the treatment of diabetes.

The following examples are provided to further define the invention without, however, limiting the invention to the particulars of these examples.

### EXAMPLE 1

#### Culture of HGT-1 and COS-7 cells

HGT-1 cells (cell line Cl.19A, a human gastric carcinoma cell line) were obtained from Dr. C. L. Laboisie and cultured as described (Laboisie *et al.*, *Cancer Research* **42**, 1541-1528 (1982)). COS-7 cells were cultured in a manner identical to HGT-1 cells.

### EXAMPLE 2

#### Cloning of a partial cDNA human GLP-1 receptor clone

Poly A<sup>+</sup> RNA was isolated from HGT-1 cells using the Fast-Track system (Invitrogen). cDNA was prepared from 5 µg of HGT-1 poly A<sup>+</sup> RNA by simultaneous priming with random hexanucleotides and oligo dT primers using the Riboclone cDNA synthesis system (Promega). HGT-1 cDNA and degenerate primers based on the rat cDNA sequence (B. Thorens, *Proceedings of the National Academy of Sciences U.S.A* **89**, 8641-8645 (1992)) were mixed in a PCR reaction to amplify a partial fragment of the human GLP-1 receptor cDNA (Figure 1, underlined). The protocol is described below:

#### Degenerate PCR

5 µl 10x PCR buffer from Boehringer Mannheim Biochemicals (BMB).  
4 µl 2.5 µM each stock dATP, dCTP, dGTP and dTTP 2 µl HGT-1 cDNA  
1 µl 20 µM primer [5' ATG CA(AG) TA(CT) TG(CT) GTN GC 3';  
SEQ ID NO:5]

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1 µl 20 uM primer [5' AT(AG) TCN GT(AC) TT(AG) CAC AT 3';  
SEQ ID NO:6]  
0.25 µl (2 units) Amplitaq DNA polymerase (Cetus)  
36.75 µl water

Reaction conditions: 40 cycles at 95°C, 1 min.; 45°C, 0.5 min.; 72°C,  
1 min.

The predominant PCR product, a 337 base pair (bp) DNA  
fragment (hGLP- fragment 1) was cloned into plasmid pCR II using the  
TA cloning kit (Invitrogen) and transformed into *Escherichia coli*.  
INVαF'. Plasmid DNA was isolated and DNA sequence determined by  
the dideoxy chain termination method.

### EXAMPLE 3

PCR amplification, cloning, and sequencing of a cDNA encoding the  
complete human GLP-1 receptor

Poly A<sup>+</sup> RNA was isolated from HGT-1 cells using the  
Fast-Track system (Invitrogen). cDNA was prepared from 5 µg of  
HGT-1 poly A<sup>+</sup> RNA by simultaneous priming with random  
hexanucleotides and oligo dT primers using the Riboclone cDNA  
synthesis system (Promega).

The cDNA was ligated with non-palindromic BST XI  
linkers (Invitrogen). Excess linkers were removed by gel-filtration  
over a cDNA sizing column (Gibco-BRL). For the PCR-RACE  
protocols the cDNA was ligated into plasmid pcDNA I (Invitrogen)  
after restriction with BST XI. A series of primers were made to  
sequences in hGLP-fragment 1 (Example 2) and to sequences in pcDNA  
I. PCR was performed as follows:

5 µl 10x PCR buffer (BMB)  
4 µl 2.5 µM each stock dATP, dCTP, dGTP and dTTP  
2 µl HGT-1 cDNA

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1 µl 20 µM primer from hGLP-fragment 1  
1 µl 20 µM primer from pcDNA 1  
0.25 µl (2 units) Amplitaq DNA polymerase  
36.75 µl water

5

Reaction conditions: 35 cycles at 95°C, 1 min.; 55°C, 0.5 min.; 72°C, 1 min.

10 Aliquots of the PCR reactions were cloned into plasmid pCR II using the TA cloning kit (Invitrogen) and transformed into *E. coli*. INVαF'. Human GLP-1 receptor specific fragments were identified by filter hybridization using [<sup>32</sup>P] labeled hGLP-fragment 1 as a probe. The following hybridization conditions were employed:

15 5X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate)  
5X Denharts solution (1% Ficoll, 1% polyvinylpyrrolidone)  
100 µg/ml salmon sperm DNA  
50% formamide  
Hybridize overnight at 42°C

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Filters were washed 2 times in 1X SSC, 0.1% SDS at room temperature for 10 min. each, then 2 times in 0.1X SSC, 0.1% SDS for 20 min. Positive clones were identified by autoradiography. DNA sequence of positive clones was obtained by the dideoxy chain  
25 termination method. Using this procedure clones containing DNA sequence from either the 5' or 3' untranslated regions were obtained.

A single molecule encoding the entire hGLP-1 receptor sequence was obtained by amplification using the polymerase chain reaction. The following conditions were employed:

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5 µl 10x PCR Pfu polymerase buffer (Stratagene).  
4 µl 2.5 µM each stock dATP, dCTP, dGTP and dTTP  
2 µl HGT-1 cDNA  
1 µl 20 uM primer 5' TGGTGGATTCCTGAACTCC 3'; SEQ ID NO:7

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1 µl 20 uM primer 5' CCTGTGGTTTCACAAGAAGC 3'; SEQ ID  
NO:8  
1 µl (5 units) Pfu polymerase (Stratagene)  
5 µl dimethylsulfoxide  
5 29 µl water

Reaction mixtures were heated at 96°C for 5 min. and then 35 thermal  
cycles performed: 1 min. at 95°C, 0.5 min. at 55°C, 2 min. at 72°C.

10 Aliquots of the PCR reactions were blunt end cloned into  
plasmid pCR-Script SK<sup>+</sup> (Stratagene) and transformed into *E. coli*  
XL-1 Blue. Human GLP-1 receptor specific fragments were identified  
by filter hybridization using [<sup>32</sup>P] labeled hGLP-fragment 1 as a probe.  
The hybridization conditions employed were those described in  
15 Example 3.

Filters were washed 2 times in 1X SSC, 0.1% SDS at room  
temperature for 10 min. each, then 2 times in 0.1X SSC, 0.1% SDS for  
20 min. Positive clones were identified by autoradiography. DNA  
sequence of positive clones was obtained by the dideoxy chain  
20 termination method. The DNA sequence obtained is shown in Figure 1.

#### EXAMPLE 4

##### Expression of the cloned human GLP-1 receptor

25 COS-7 cells were transfected by electroporation with the  
human GLP-1 receptor cDNA subcloned into the eukaryotic expression  
vector pcDNA I/neo (Invitrogen). Cells were harvested after 60-72 h.  
Membranes containing the expressed receptor protein were prepared as  
described (C. D. Strader *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4384-  
30 4388 (1987). Membranes prepared from the COS-7 cells transfected  
with the vector containing the human GLP-1 receptor cDNA  
specifically bound the GLP-1 receptor agonist [<sup>125</sup>I] GLP-1 (7-36)  
amide (Figure 3). Membranes prepared from cells transfected with the  
vector alone did not specifically bind [<sup>125</sup>I] GLP-1 (7-36) amide,

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proving the expression of the human GLP-1 receptor. As shown in Figure 3, GLP-1 (7-36) amide inhibits the binding of [<sup>125</sup>I] GLP-1 (7-36) amide to the receptor with an IC<sub>50</sub> of 4 nM. Glucagon, gastric inhibitory peptide, and secretin inhibit [<sup>125</sup>I] GLP-1 (7-36) amide binding with a potency at least a 100-fold lower, consistent with the identification of the receptor as a GLP-1 receptor.

Binding reactions were performed in a final volume of 200 µl of PBS (10 mM sodium phosphate, 1 mM potassium phosphate, 2.7 mM potassium chloride, 137 mM sodium chloride, pH 7.0 ) under the following conditions:

10-25 µg COS-7 membranes prepared from transfected cells  
0.1% bovine serum albumin  
50 pM [<sup>125</sup>I] GLP-1 (7-36) amide  
0-1 µM GLP-1 (7-36) amide (or the other compounds listed in the legend to Figure 3)

Membranes were incubated at room temperature with shaking for one hour. Membranes were harvested on GF/C filters (Whatman) that had been presoaked in 0.5% polyethylenimine/0.1% BSA. The filters were washed three times with ice-cold PBS and bound radioactivity determined by gamma counting. Data were analyzed using the Inplot program (Graphpad Software).

The human GLP-1 receptor is functionally coupled to adenylyl cyclase in transiently transfected COS-7 cells (Figure 4). Incubation of COS-7 cells expressing the human GLP-1 receptor with GLP-1 (7-36) amide leads to a 4-fold increase in cyclic AMP (cAMP) over basal levels. Under identical assay conditions mock transfected COS cells show no significant increase in cAMP over basal levels. GLP-1 (7-36) amide stimulates cAMP accumulation with an EC<sub>50</sub> of 25 pM. Glucagon also stimulates cAMP accumulation in COS-7 cells transfected with the human GLP-1 receptor but with a 200-fold decrease in potency compared with GLP-1 (7-36) amide (Figure 4). The decreased potency for glucagon is consistent with its acting via the

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human GLP-1 receptor. Stimulation of cAMP accumulation in intact COS-1 cells that had been transfected with the hGLP-1 receptor assays were carried out in 120 µl volume of ACC (75 mM Tris pH 7.4, 250 mM sucrose, 12.5 mM magnesium chloride, 1.5 mM ethylenediamine-tetraacetic acid (EDTA), 0.1 mM of the phosphodiesterase inhibitor Ro-201724) containing the following additions:

50,000 COS-7 cells transfected with the human GLP-1 receptor expression construct; and  
0-100 µM GLP-1 (7-36) amide or glucagon

Reaction mixtures were incubated for 45 min. at room temperature with shaking. Reactions were terminated by boiling for 3-5 min. cAMP was determined by radioimmunoassay.

#### EXAMPLE 5

Screening Assay: Glucagon-like peptide-1 receptor mediated increase in intracellular cAMP

Transfected cells expressing recombinant human GLP-1 receptor may be used to identify compounds that are agonists for it. This is done by incubating cells with test compounds in range of concentrations such that an ED50 for the test compound is obtained. The ED50 is defined as the concentration of compound which increases intracellular cyclic AMP (cAMP) to 50% of the maximal quantity of cAMP produced by interaction of the test compound with the human GLP-1 receptor. These reactions are carried out in 120 µl volume of ACC containing the following additions:

50,000 COS-7 cells transfected with the human GLP-1 receptor; and  
Various concentration of test compounds.

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Reaction mixtures were incubated for 45 min. at room temperature with shaking. Reactions were terminated by boiling for 3-5 min. cAMP was determined by radioimmunoassay.

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- 14 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

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- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
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  - (B) FILING DATE:
  - (C) CLASSIFICATION:
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1567 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA



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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGGTGAATT CCTGAACTCC CCGCCATGGC CGGCGCCCCC GGCCCGCTGC GCCTTGCGCT 60  
GCTGCTGCTC GGGATGGTGG GCAGGGCCCG CCCCCGCCCC CAGGGTGCCA CTGTGTCCCT 120  
CTGGGAGACG GTGCAGAAAT GGCGAGAATA CCGACGCCAG TGCCAGCGCT CCCTGACTGA 180  
GGATCCACCT CCTGCCACAG ACTTGTTCCTG CAACCGGACC TTCGATGAAT ACGCCTGCTG 240  
GCCAGATGGG GAGCCAGGCT CGTTCGTGAA TGTCAGCTGC CCCTGGTACC TGCCCTGGGC 300  
CAGCAGTGTG CCGCAGGGCC ACGTGTACCG GTTCTGCACA GCTGAAGGCC TCTGGCTGCA 360  
GAAGGACAAC TCCAGCCTGC CCTGGAGGGA CTTGTCTGGAG TGCGAGGAGT CCAAGCGAGG 420  
GGAGAGAAGC TCCCCGGAGG AGCAGCTCCT GTTCCTCTAC ATCATCTACA CGGTGGGCTA 480  
CGCACTCTCC TTCTCTGCTC TGGTTATCGC CTCTGCGATC CTCCTCGGCT TCAGACACCT 540  
GCACTGCACC AGGAACTACA TCCACCTGAA CCTGTTTGCA TCCTTCATCC TGCGAGCATT 600  
GTCCGTCTTC ATCAAGGACG CAGCCCTGAA GTGGATGTAT AGCACAGCCG CCCAGCAGCA 660  
CCAGTGGGAT GGGCTCCTCT CCTACCAGGA CTCTCTGAGC TGCCGCCTGG TGTCTCTGCT 720  
CATGCAGTAC TGTGTGGCGG CCAATTACTA CTGGCTCTTG GTGAGGGCG TGTACCTGTA 780  
CACACTGCTG GCCTTCTCGG TCTTATCTGA GCAATGGATC TTCAGGCTCT ACGTGAGCAT 840  
AGGCTGGGGT GTTCCCCTGC TGTTTGTTGT CCCCTGGGGC ATTGTCAAGT ACCTCTATGA 900  
GGACGAGGGC TGCTGGACCA GGAAGTCCAA CATGAACTAC TGGCTCATTA TCCGGCTGCC 960  
CATCTCTTTT GCCATTGGGG TGAAGTTCCT CATCTTTGTT CGGGTCATCT GCATCGTGGT 1020  
ATCCAAACTG AAGGCCAATC TCATGTGCAA GACAGACATC AAATGCAGAC TTGCCAAGTC 1080  
CACGCTGACA CTCATCCCCC TGCTGGGGAC TCATGAGGTC ATCTTTGCCT TTGTGATGGA 1140  
CGAGCACGCC CGGGGGACCC TGCGCTTCAT CAAGCTGTTT ACAGAGCTCT CCTTCACCTC 1200  
CTTCCAGGGG CTGATGGTGG CCATATTATA CTGCTTTGTC AACAATGAGG TCCAGCTGGA 1260  
ATTTTCGGAAG AGCTGGGAGC GCTGGCGGCT TGAGCACTTG CACATCCAGA GGGACAGCAG 1320  
CATGAAGCCC CTCAAGTGTC CCACCAGCAG CCTGAGCAGT GGAGCCACGG CGGGCAGCAG 1380  
CATGTACACA GCCACTTGCC AGGCCTCCTG CAGCTGAGAC TCCAGCGCCT GCCCTCCCTG 1440  
GGGTCCTTGC TGCAGGCCCG GTGGCCAATC CAGGAGAAGC AGCCTCCTAA TTTGATCACA 1500  
GTGGCGAGAG GAGAGGAAAA ACGATCGCTG TGAAAATGAG GAGGATTGCT TCTTGTGAAA 1560  
CCACAGG 1567

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## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 463 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Gly Ala Pro Gly Pro Leu Arg Leu Ala Leu Leu Leu Leu Gly  
 1 5 10 15

Met Val Gly Arg Ala Gly Pro Arg Pro Gln Gly Ala Thr Val Ser Leu  
 20 25 30

Trp Glu Thr Val Gln Lys Trp Arg Glu Tyr Arg Arg Gln Cys Gln Arg  
 35 40 45

Ser Leu Thr Glu Asp Pro Pro Pro Ala Thr Asp Leu Phe Cys Asn Arg  
 50 55 60

Thr Phe Asp Glu Tyr Ala Cys Trp Pro Asp Gly Glu Pro Gly Ser Phe  
 65 70 75 80

Val Asn Val Ser Cys Pro Trp Tyr Leu Pro Trp Ala Ser Ser Val Pro  
 85 90 95

Gln Gly His Val Tyr Arg Phe Cys Thr Ala Glu Gly Leu Trp Leu Gln  
 100 105 110

Lys Asp Asn Ser Ser Leu Pro Trp Arg Asp Leu Ser Glu Cys Glu Glu  
 115 120 125

Ser Lys Arg Gly Glu Arg Ser Ser Pro Glu Glu Gln Leu Leu Phe Leu  
 130 135 140

Tyr Ile Ile Tyr Thr Val Gly Tyr Ala Leu Ser Phe Ser Ala Leu Val  
 145 150 155 160

Ile Ala Ser Ala Ile Leu Leu Gly Phe Arg His Leu His Cys Thr Arg  
 165 170 175

Asn Tyr Ile His Leu Asn Leu Phe Ala Ser Phe Ile Leu Arg Ala Leu  
 180 185 190

Ser Val Phe Ile Lys Asp Ala Ala Leu Lys Trp Met Tyr Ser Thr Ala  
 195 200 205

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Ala	Gln	Gln	His	Gln	Trp	Asp	Gly	Leu	Leu	Ser	Tyr	Gln	Asp	Ser	Leu	210	215	220	
Ser	Cys	Arg	Leu	Val	Phe	Leu	Leu	Met	Gln	Tyr	Cys	Val	Ala	Ala	Asn	225	230	235	240
Tyr	Tyr	Trp	Leu	Leu	Val	Glu	Gly	Val	Tyr	Leu	Tyr	Thr	Leu	Leu	Ala	245	250	255	
Phe	Ser	Val	Leu	Ser	Glu	Gln	Trp	Ile	Phe	Arg	Leu	Tyr	Val	Ser	Ile	260	265	270	
Gly	Trp	Gly	Val	Pro	Leu	Leu	Phe	Val	Val	Pro	Trp	Gly	Ile	Val	Lys	275	280	285	
Tyr	Leu	Tyr	Glu	Asp	Glu	Gly	Cys	Trp	Thr	Arg	Asn	Ser	Asn	Met	Asn	290	295	300	
Tyr	Trp	Leu	Ile	Ile	Arg	Leu	Pro	Ile	Leu	Phe	Ala	Ile	Gly	Val	Asn	305	310	315	320
Phe	Leu	Ile	Phe	Val	Arg	Val	Ile	Cys	Ile	Val	Val	Ser	Lys	Leu	Lys	325	330	335	
Ala	Asn	Leu	Met	Cys	Lys	Thr	Asp	Ile	Lys	Cys	Arg	Leu	Ala	Lys	Ser	340	345	350	
Thr	Leu	Thr	Leu	Ile	Pro	Leu	Leu	Gly	Thr	His	Glu	Val	Ile	Phe	Ala	355	360	365	
Phe	Val	Met	Asp	Glu	His	Ala	Arg	Gly	Thr	Leu	Arg	Phe	Ile	Lys	Leu	370	375	380	
Phe	Thr	Glu	Leu	Ser	Phe	Thr	Ser	Phe	Gln	Gly	Leu	Met	Val	Ala	Ile	385	390	395	400
Leu	Tyr	Cys	Phe	Val	Asn	Asn	Glu	Val	Gln	Leu	Glu	Phe	Arg	Lys	Ser	405	410	415	
Trp	Glu	Arg	Trp	Arg	Leu	Glu	His	Leu	His	Ile	Gln	Arg	Asp	Ser	Ser	420	425	430	
Met	Lys	Pro	Leu	Lys	Cys	Pro	Thr	Ser	Ser	Leu	Ser	Ser	Gly	Ala	Thr	435	440	445	
Ala	Gly	Ser	Ser	Met	Tyr	Thr	Ala	Thr	Cys	Gln	Ala	Ser	Cys	Ser	450	455	460		

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 19 base pairs  
 (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGTGGATTC CTGAACTCC

19

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTGTGGTTT CACAAGAAGC

20

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGCAAGTAC TTGCTGTGCN

20

## (2) INFORMATION FOR SEQ ID NO:6:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAGTCNGTAC TTAGCACAT

20

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGTGGATTC CTGAACTCC

19

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTGTGGTTT CACAAGAAGC

20

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WHAT IS CLAIMED IS:

- 5 1. An isolated and purified DNA molecule consisting essentially of DNA encoding a human glucagon-like 1 peptide receptor.
- 10 2. The DNA molecule of Claim 1, wherein the DNA coding for the receptor is operably linked to regulatory sequences such that the receptor may be expressed upon introduction into a prokaryotic or eukaryotic cell.
3. The human glucagon-like 1 peptide receptor encoded by the DNA molecule of Claim 1.
- 15 4. A cell containing the DNA molecule of Claim 1, the cell expressing a cloned human glucagon-like 1 peptide receptor.
- 20 5. A method for identifying compounds which specifically bind to a human glucagon-like 1 peptide receptor comprising the steps of:
  - 25 (a) cloning the human glucagon-like 1 peptide receptor;
  - (b) splicing the the cloned glucagon-like 1 peptide receptor into an expression vector to produce a construct such that the glucagon-like 1 peptide receptor is operably linked to transcription and translation signals sufficient to induce expression of the receptor upon introduction of the construct into a prokaryotic or eukaryotic cell;
  - 30 (c) introducing the construct into a prokaryotic or eukaryotic cell which does not express a human receptor in the absence of the introduced construct;

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- 5
- (d) incubating cells or membranes isolated from cells produced in Step (c) with a quantifiable compound known to bind to human glucagon-like 1 peptide receptor, and subsequently adding test compounds at a range of concentrations so as to compete the quantifiable compound from the receptor; and
- 10
- (e) calculating the relative binding of the test compound to the cells or membranes of Step (d).

6. The compounds identified by the method of Claim 5.

15

7. A method of alleviating the effects of diabetes which comprises administering a pharmaceutically effective amount of a compound which specifically binds to the human GLP-1 receptor.

20

8. The DNA molecule of Claim 1 having the nucleic acid sequence shown in Figure 1.

25

9. A purified human glucagon-like 1 peptide receptor encoded by the DNA molecule of Claim 8, the purified receptor having the amino acid sequence shown in Figure 1.

30

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1 1gg1gg0attcctgaactccccgccATGGCCGGCGCCCCGGCCCGCTGCGCCTTGCGCT  
M A G A F G P L R L A L  
61 GCTGCTGCTCGGGATGGTGGGCAGGGCCGGCCCCCGCCCCAGGGTGCCACTGTGTCCCT  
L L L G M V G R A G F R F Q G A T V S L  
121 CTGGGAGACGGTGCAGAAATGGCGAGAATACCGACGCCAGTGCCAGCGCTCCCTGACTGA  
W E T V Q K W R E Y R R Q C Q R S L T E  
181 GGATCCACCTCCTGCCACAGACTTGTTCGCAACCGGACCTTCGATGAATACGCCTGCTG  
D F P F A T D L F C N R T F D E Y A C W  
241 GCCAGATGGGGAGCCAGGCTCGTTCGTGAATGTCAGCTGCCCCCTGGTACCTGCCCTGGGC  
F D G E P G S F V N V S C F W Y L P W A  
301 CAGCAGTGTCCCGCAGGGCCACGTGTACCGTTCTGCACAGCTGAAGGCCTCTGGCTGCA  
S S V P Q G N V Y R F C T A E G L W L Q  
361 GAAGGACAACTCCAGCCTGCCCTGGAGGGACTTGTGGAGTCCGAGGAGTCCAAGCGAGG  
K D N S S L P W R D L S E C E E S K R G  
421 GGAGAGAAGCTCCCCGGAGGAGCAGCTCCTGTTCTCTACATCATCTACACGGTGGGCTA  
E R S S P E E Q L L F L Y I I Y T V G Y  
481 CGCACTCTCCTTCTCTGCTCTGGTTATCGCCTCTGCGATCCTCCTCGGCTTCAGACACCT  
A L S F S A L V I A S A I L L G F R N L  
541 GCACTGCACCAGGA ACTACATCCACCTGAACCTGTTTGCATCCTTCATCCTCGGAGCATT  
N C T R N Y I N L N L F A S F I L R A L

FIG.1A

SUBSTITUTE SHEET (RULE 26)



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601 GTCCGTCTTCATCAAGGACGCAGCCCTGAAGTGGATGTATAGCACAGCCGCCACGAGCA  
S V F I K D A A L K W M Y S T A A Q Q N  
661 CCAGTGGGATGGGCTCCTCTCCTACCAGGACTCTCTGAGCTGCCGCCTGGTGTCTTCTGCT  
Q W D G L L S Y Q D S L S C R L V F L L  
721 CATGCAGTACTGTGTGGCGGCCAATTACTACTGGCTCTTGGTGGAGGGCGTGACCTGTA  
M Q Y C V A A N Y Y N L L V E G V Y L Y  
781 CACACTGCTGGCCTTCTCGGTCTTATCTGAGCAATGGATCTTCAGGCTCTACGTGAGCAT  
T L L A F S V L S N Q W I F R L Y V S I  
841 AGGCTGGGGTGTTCCTGCTGTTTGTGTCCCTGGGGCATTGTCAAGTACCTCTATGA  
G W G V F L L F V V P W G I V K Y L Y E  
901 GGACGAGGGCTGCTGGACCAGGAACTCCAACATGAACTACTGGCTCATTATCCGGCTGCC  
D E G C W T R N S N M N Y N L I I R L P  
961 CATTCTCTTTGCCATTGGGGTGAAGTCTCTCATCTTTGTTCCGGTCATCTGCATCGTGGT  
I L F A I G V N F L I F V R V I C I V V  
1021 ATCCAAACTGAAGGCCAATCTCATGTGCAAGACAGACATCAAATGCAGACTTGCCAAGTC  
S K L K A N L M C K T D I K C R L A K S  
1081 CACGCTGACACTCATCCCCCTGCTGGGACTCATGAGGTCATCTTGCCTTTGTGATGGA  
T L T L I P L L G T N E V I F A F V M D  
1141 CGAGCACGCCCCGGGGGACCCTGCGCTTCATCAAGCTGTTTACAGAGCTCTCCTTCACCTC  
E N A R G T L R F I K L F T E L S F T S

FIG.1B

SUBSTITUTE SHEET (RULE 26)

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1201 CTTCAGGGGCTGATGGTGGCCATATTATACTGCTTTGTCAACAATGAGGTCCAGCTGGA  
F Q G L M V A I L Y C F V N N E V Q L E  
1261 ATTCGGAAGAGCTGGGAGCGCTGGCGGCTTGAGCACTTGCACATCCAGAGGGACAGCAG  
F R K S W E R W R L E N L N I Q R D S S  
1321 CATGAAGCCCCTCAAGTGTCCCACCAGCAGCCTGAGCAGTGGAGCCACGGCGGGCAGCAG  
M K P L K C P T S S L S S G A T A G S S  
1381 CATGTACACAGCCACTTGCCAGGCCTCCTGCAGCtgagactccagcgccctgccctccctg  
M Y T A T C Q A S C S \* SEQ ID NO: 2  
1441 gggtccttgctgcaggccgggtggccaatccaggagaagcagcctcctaattlgatcaca  
1501 gtggcgagaggagaggaaaaacgatcgctgtgaaaatgaggaggattgcttcttgtgaaa  
1561 ccacagg SEQ ID NO: 1

FIG.1C

FIG. 2

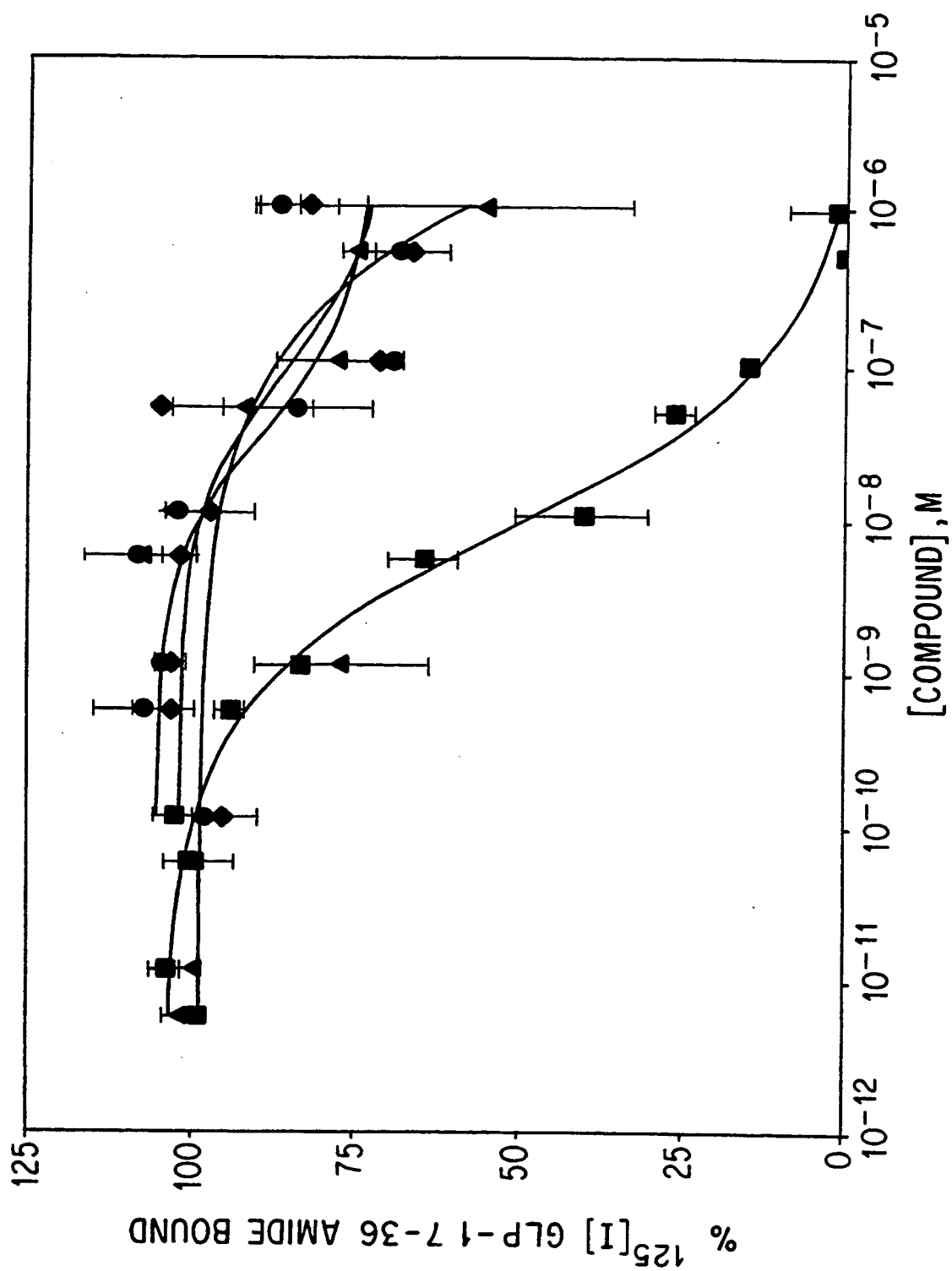


FIG. 3

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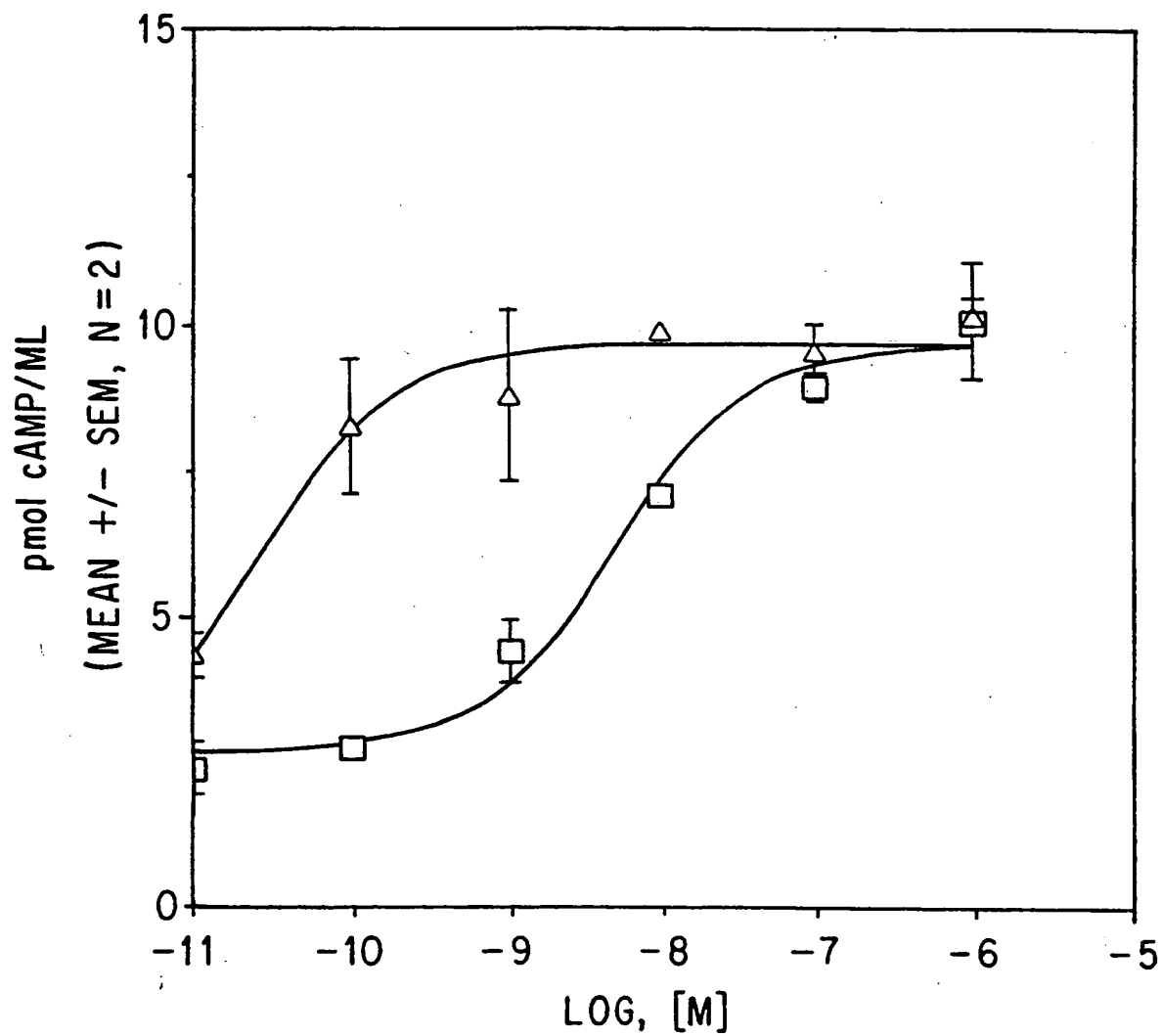


FIG. 4

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08913

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/12, 15/63, 5/10; C12Q 1/02

US CL : 536/23.5; 435/320.1, 240.2, 7.21; 530/350, 395

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/320.1, 240.2, 7.21; 530/350, 395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Diabetes, Volume 42, Number 8, issued 1993, M. Stoffel et al., "Human Glucagon-Like Peptide-1 Receptor Gene", pages 1215-1218, especially the abstract.	1
Y	Proceedings of the National Academy of Sciences USA, Volume 89, issued September 1992, B. Thorens, "Expression cloning of the pancreatic $\beta$ cell receptor for the gluco-incretin hormone glucagon-like peptide 1", pages 8641-8645, especially page 8641, col. 1; page 8642; and page 8644.	1-5, 8, 9
Y	M. A. Innis et al., "PCR Protocols: A Guide to Methods and Applications", published 1990 by Academic Press, Inc. (New York), chapter 11, pages 84-91, see the entire chapter.	1-5, 8, 9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* & * document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

03 OCTOBER 1994

Date of mailing of the international search report

16 NOV 1994

Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08913

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular Endocrinology, Volume 4, Number 2, issued February 1990, M. Birnbaumer et al., "Development and Characterization of a Mouse Cell Line Expressing the Human V2 Vasopressin Receptor Gene", pages 245-254, especially page 251, col. 2.	1-5, 8, 9

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08913

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-5, 8, and 9

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.



**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

USPTO-APS, Medline, Scisearch, Biosis, Embase, Derwent WPI

Search terms: Glucagonlike, glucagon()like; bind?, receptor, ligand; clon?, recombinant?, cDNA; human  
IgSuite Sequence Databases

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

- I. Claims 1-5, 8, and 9, drawn to human glucagon-like peptide I receptor (GLPR) DNA, vectors, transformed cells, proteins, and screening assays employing them.
- II. Claims 6 and 7, drawn to compounds identified using the screening assays of group I and to therapeutic methods employing such compounds.

The inventions are related because the compounds of group II may be identified using the compositions and assays of group I. However, they are distinct because the group II compounds may also be identified with screening assays employing natural tissue or cellular isolates expressing native GLPR. The invention of group II thus does not require the compositions and methods of group I for its practice. For these reasons, the inventions are considered not to be so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single general inventive concept.

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